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Stable lines of transgenic zebrafish exhibit reproducible patterns of transgene expression.

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To study the frequency of germ-line transformation and to examine the reproducibility of tissue-specific transgene expression, we produced several lines of transgenic zebrafish expressing a recombinant chloramphenicol acetyltransferase (CAT) gene. Supercoiled plasmids containing both Rous sarcoma virus and SV-40 promoter sequences upstream of the CAT coding region were injected into zebrafish embryos prior to first cleavage. CAT activity could be detected in batches of injected embryos as early as 8 h and up to at least 12 days post-fertilization. Approximately 18% of injected fish raised to maturity exhibited CAT activity in their fins, and approximately 5% of injected fish became stable germ-line transformants. Breeding studies indicated that although transgenic founder fish were frequently germ-line mosaics, transgenic individuals of subsequent generations were fully hemizygous for the transgene marker. The transgenes present in the F1 progeny of four independent lines were relatively well expressed in fin and skin, while lower levels of expression were observed in heart, gill and muscle. Little or no CAT expression was observed in the brain, liver and gonad. A monoclonal antibody directed against the CAT gene product consistently revealed variegated patterns of CAT expression in ectodermally derived fin epidermal cells in three of these lines. These results show that it is possible to efficiently produce stable germ-line transformants of the zebrafish and to observe reproducible tissue-specific patterns of transgene expression in this organism. Possible mechanisms for the variegated expression observed within tissues are also considered.

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